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EXPERIMENTS IN THE CONTROL OF RHIZOCTONIA DAMPING-OFF OF CITRUS SEEDLINGS[1, 2]

R. WEINDLING[3] AND H. S. FAWCETT[4]

INTRODUCTION

Rhizoctonia solani Kühn has been reported from various localities as the most important fungus associated with the damping-off disease of citrus seedlings.(5, 19, 20, 21, 25) During the last four years this fungus has been constantly isolated from diseased seedlings of sweet orange, grapefruit, and sour orange which were collected in seed beds from various sections of southern California. In the present experiments, the high virulence of the pathogen in sterilized and natural soils has been repeatedly verified. There is an indication from field and greenhouse observations that sour orange is not quite so susceptible to the attack of the fungus as sweet orange.

The disease is readily recognized in the seed bed. The dead seedlings with withered leaves usually remain standing about the margins of the bare areas which mark the centers of infection. At the margins of these areas may be found plants with lesions and apparently healthy seedlings with strands of the fungus hanging like spider webs around the base of the stem. In general, nurserymen are able to avoid damping-off or to keep it within reasonable limits by good cultural practices, such as are discussed by Fawcett and Lee.(5) In some seasons and in some sections, however, the losses due to damping-off have been excessive. In 1934, for instance, 50 to 60 per cent of the seedlings in some beds were affected.

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[2] Paper No. 324, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Technical Assistant in Plant Pathology, Citrus Experiment Station; resigned June 30, 1935.

[4] Professor of Plant Pathology in the Citrus Experiment Station and Graduate School of Tropical Agriculture and Plant Pathologist in the Experiment Station.

In this paper account will be given of two field experiments in which considerable control of damping-off was obtained by acidifying the surface layer of the soil by the application of acid peat moss or aluminum sulfate. Some details of laboratory and greenhouse experiments will also be presented.[31] These experiments had been planned primarily to furnish a basis for control methods in the field, but they also threw light on the reasons why control of *Rhizoctonia* damping-off is obtained by acidification of the top soil.

LABORATORY AND GREENHOUSE EXPERIMENTS IN BIOLOGICAL CONTROL OF DAMPING-OFF

FACTORS INVOLVED

Hartley and his collaborators[10, 11] demonstrated several years ago that saprophytic soil fungi may play a rôle in decreasing damping-off of conifer seedlings. It was suggested that this might be due to their competition in the soil with the pathogenic organisms and perhaps to their antagonistic effects upon these organisms. Since the discovery by Weindling[28] of the parasitic action of *Trichoderma* on *Rhizoctonia solani* and other soil fungi, two sets of investigators[2, 3] have obtained positive results with *Trichoderma* spp. for biological control of pathogenic soil fungi. In our first experiments of this kind, application of *Trichoderma* had given good protection to seedlings against *Rhizoctonia* in some cases and little or none in others. These contradictory results were clarified later by a research in the fundamental aspects of the interaction between *Trichoderma* and *Rhizoctonia*. It was shown[29] that the parasitic action of the strain of *Trichoderma* used is favored by a strongly acid reaction of the culture medium, and decreases as the medium becomes less acid. Similar effects were obtained in our laboratory experiments with soils autoclaved at 120° C three times for a period of 1 hour each.

Sterilized soils have been used by numerous investigators to demonstrate that plant diseases caused by certain soil-borne plant pathogens may be prevented or reduced by inoculating these soils with antagonistic organisms or with portions of unsterilized soil.[12, 18, 24] It has been claimed that the development of fungi in sterilized soil is entirely unlike their growth in natural soil, and that it is much more comparable to artificial growth in culture media.[17] Nevertheless, the data from our work with sterilized soil proved to be of value as a basis for planning and interpreting the work with nonsterilized soil.

EFFECT OF pH AND OTHER FACTORS IN LABORATORY EXPERIMENTS

The following are representative of this type of experiment. As a rule, each treatment was given to three containers. Since inoculation with *Trichoderma* alone did not differ from the controls in which neither *Rhizoctonia* nor *Trichoderma* was used, this treatment was omitted in most of the later experiments. Usually the number of seeds planted was twelve per container.

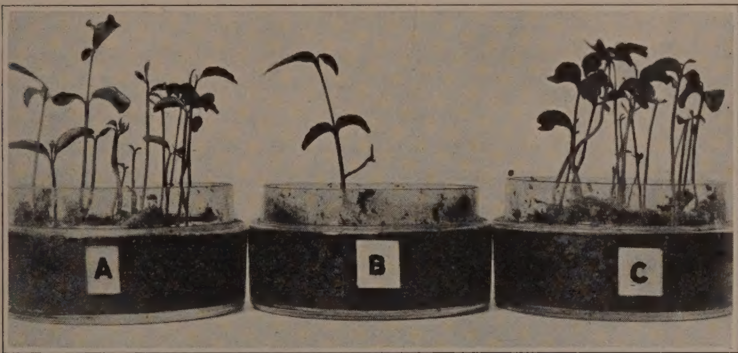

Fig. 1.—Sweet-orange seedlings in sterilized soil. *A*, Check; *B*, *Rhizoctonia* inoculated in soil layer in bottom of jar; *C*, *Rhizoctonia* as in *B*, plus *Trichoderma* in top layer of peat.

In the first test, petri dishes containing peat moss (pH 4.0) were autoclaved and sown with citrus seeds taken aseptically from the fruits. When *Rhizoctonia* alone was inoculated, all germinating seedlings were killed. But if *Trichoderma* spores were at the same time mixed with the peat or sprayed onto the seed, none of the seedlings were attacked.

In another experiment the acid peat moss was adjusted to a series of pH values by the addition of potassium hydroxide. Portions of peat of each acidity were sterilized in pint Mason jars. At the original acidity (pH 4.0) the protection of the seedlings from *Rhizoctonia* by adding *Trichoderma* was as good as in the first test, previously described. At pH 4.5 to 4.7, only 68 per cent control was obtained, and at pH 5.4 to 5.6 and at higher pH values, the loss due to rotting of the germinating seed and to damping-off was as complete as with *Rhizoctonia* alone. In another set with a weaker inoculation of *Rhizoctonia,* the *Trichoderma* protected 70 per cent of the seedlings at pH 4.5 and 50 per cent at pH 5.7 to 6.1, but none at neutral reaction.

Figure 1 shows an experiment with seedlings in glass jars. A mixture of sand and soil (pH 6.5) was placed in the bottom of the jars and this

was covered with an inch of peat. After sterilization, *Rhizoctonia* was inoculated in the bottom soil. The seeds and *Trichoderma* were placed in the peat. In the jars with *Rhizoctonia* alone 90 per cent of the seedlings were killed before emergence; the rest damped-off later. The dishes inoculated with *Rhizoctonia* and with spores of a pigmented culture of *Trichoderma* showed 40 and 50 per cent healthy seedlings, while a culture of *Trichoderma* with a coconut-like odor gave 78 and 95 per cent control.[5]

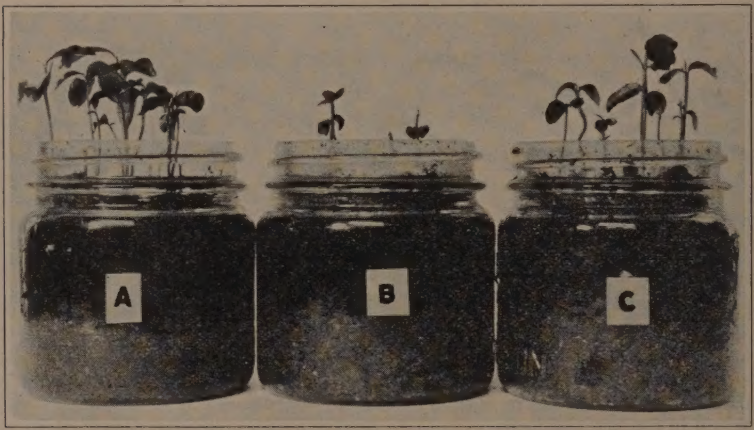

Fig. 2.—Sweet-orange seedlings in nonsterilized soil. *A*, Check; *B*, *Rhizoctonia* inoculated in soil layer in bottom of jar; *C*, *Rhizoctonia* as in *B*, plus *Trichoderma* in top layer of peat.

An experiment similar to the last one mentioned is represented in figure 2. However, these jars with soil and peat were not sterilized. The seed was covered with a mixture of sand and peat. The spores of *Trichoderma* were suspended in a 1 per cent glycerine solution, on which *Trichoderma* grows much better than does *Rhizoctonia*. This fact was established in an experiment in which all other carbon sources tested produced a much denser growth of *Rhizoctonia* than of *Trichoderma*. In *Trichoderma*-inoculated jars, 92 per cent of the seedlings were not affected by damping-off. In the jars with *Rhizoctonia* alone, 22 per cent of the seedlings survived. Abundant sporulation of *Trichoderma* was found, however, on the soil in these jars. A similar effect was noted in an experiment with sterilized acid peat, where insufficient care had been taken to prevent the jars with *Rhizoctonia* alone from being contaminated by the air-borne spores of *Trichoderma*. If other organisms were

[5] The first percentage in each case refers to soil inoculation with *Trichoderma*, the second to seed inoculation.

kept away from sterilized soils, *Rhizoctonia* inoculation would always result in the death of all seedlings, mostly before emergence.

In the work reported thus far, the jars were covered and placed in an incubator at 27° C until the seeds had germinated. Then they were uncovered and held in the laboratory at room temperature. The *Rhizoctonia* was grown on wheat bran for 2 to 4 weeks, 1 to 3 cc of bran being added to the soil; the amount varied with the size of the containers used in these and the following experiments. The spore suspension of *Trichoderma* was prepared from cultures of the fungus that had grown for 10 to 20 days on glucose-potato agar. In some of the work, cultures of *Trichoderma lignorum* producing a coconut-like odor and cultures of *T. koningi* were used in addition to the pigmented cultures of *T. lignorum*. The hydrogen-ion concentrations were determined by the method proposed by Hissink.[13]

EFFECT OF pH AND OTHER FACTORS IN GREENHOUSE EXPERIMENTS

The acid peat moss had proved to be a very favorable medium for *Trichoderma*. It was used, therefore, in experiments in the greenhouse, carried out to develop methods suitable for preventing the damping-off disease under seed-bed conditions. In general, the plan was to place a sandy loam of about neutral reaction in the bottom of pots or flats, and to inoculate this soil with the *Rhizoctonia* at one spot or several. The soil was covered with a layer of peat moss which served as a bed for the seed. Grapefruit seeds were used most frequently, but sweet and sour orange were used in some cases. The number of seeds used was 25 per 5-inch pot, and 250 to 300 per flat of 2 square feet. The *Trichoderma* spore suspension was added to the peat. The controls consisted of containers with surface layers of soil or peat, *Rhizoctonia* alone being inoculated, or no fungus at all. A digest is here presented of results of a large number of experiments made in an attempt to find the best methods of procedure.

Nearly complete protection from the disease was obtained where a layer of peat, ¾-inch deep and having a reaction of pH 4.0, was placed on the soil, and when the seed was covered with an additional ¾-inch layer of peat moss inoculated with *Trichoderma*. However, when the seed was placed on the soil and covered with sand, all or nearly all of the germinating seedlings were killed by the *Rhizoctonia,* even if *Trichoderma* spores were added in abundance. The reaction of the soil was pH 6.4 to 6.7, that of the sand, pH 7.3 to 7.8. Where peat was used in the containers inoculated with *Rhizoctonia* alone, considerable damping-off sometimes developed; in other cases, however, the disease affected very few seedlings. In the above cases when only a few seedlings were affected by the introduction of *Rhizoctonia* alone with peat, *Trichoderma* could

always be easily isolated from the containers, even if they were kept as far as possible from soil purposely inoculated with this fungus. Thus, *Trichoderma* spp., even if not purposely introduced, seem to represent a group of organisms which at acid reactions of the soil prevent the *Rhizoctonia* from becoming fatal to the seedlings.

The use of any mixture of the peat with sand or soil gave poorer control than with the straight peat. It was with the mixtures of peat and

Fig. 3.—At left, grapefruit seedlings in natural soil. At right, soil cover replaced by peat 1 inch deep. Both sides inoculated with *Rhizoctonia* in subsoil at lower end of flat.

sand, however, that biological control with *Trichoderma* could be demonstrated in some experiments. In one of them the seed was covered with a mixture of peat and sand (initial pH 4.5 to 4.8). From 25 to 40 per cent control was obtained from inoculation with *Trichoderma,* inoculation with *Rhizoctonia* alone producing complete loss.

In general, the control of the disease seemed to increase with increasing acidity of the peat mixture. Changes in reaction of the soils were followed over the period of damping-off, that is, for about 14 weeks, by testing samples with the quinhydrone electrode. With smaller percentages of peat in the mixtures, the change towards a neutral reaction was, of course, more rapid. But even in the flats and pots with peat alone there was a gradual decrease of acidity, probably due to the alkalinity of the irrigation water.

Results similar to those reported thus far were also obtained with seedlings inoculated when 6 to 8 weeks old. In figure 3 is shown an example

of this type of experiment. On one side of the flat the upper inch layer of soil was replaced by *Trichoderma*-infested peat, simultaneously with the introduction of the *Rhizoctonia* inoculum in the subsoil at both sides of the partition at the lower end of the flat.

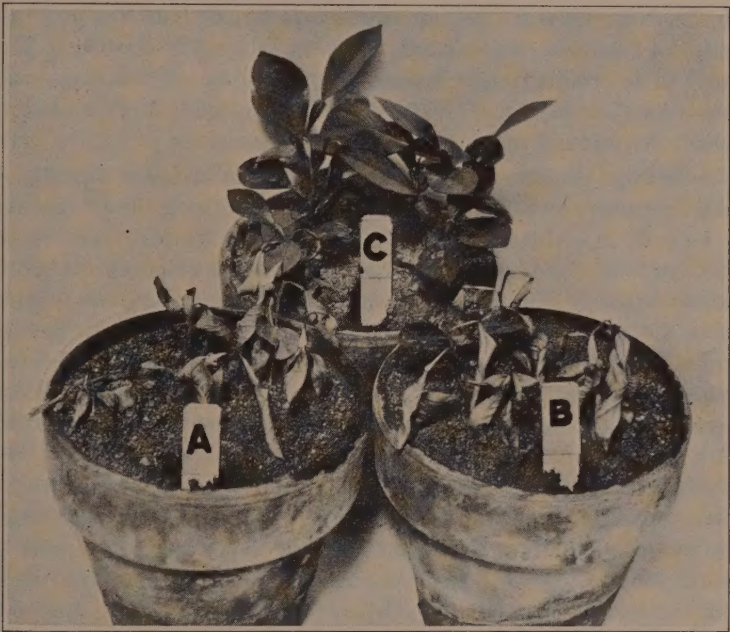

Fig. 4.—Sour-orange seedlings in natural soil. In pots *B* and *C*, the surface inch of the soil was replaced by the same soil treated with aluminum sulfate, simultaneously with *Rhizoctonia* inoculation. Initial reaction of the soil-surface layers: Pot *A*, pH 7.3; Pot *B*, pH 4.9; Pot *C*, pH 4.0.

In other experiments, acidification of the top layer of the soil was attempted by applying aluminum sulfate, which has been frequently employed as a quick and convenient means of soil acidification.[6] (10, 32) Figure 4 shows the effect on seedlings of *Rhizoctonia* inoculation made at the same time that the upper inch of a slightly alkaline soil was replaced with soil treated with aluminum sulfate to give pH 4.9 to pot *B* and pH 4.0 to pot *C*.

The same soils were also used as surface layers in flats and pots in which seeds were sown. At pH 7.3 there was a total loss, while complete control of the disease was secured at pH 4.0, and only partial control at pH 4.9. Similar results were obtained in each pH series, three pots

[6] The writers are indebted to D. E. Bliss, Citrus Experiment Station, for advice regarding methods of applying aluminum sulfate.

being inoculated with *Rhizoctonia* alone, and three with *Rhizoctonia* and *Trichoderma*. Mycelium of *Rhizoctonia* was reisolated easily from the top soil of the pots that had not received *Trichoderma* and which had a reaction of pH 7.3. It was also found in some cultures from soils of pH 4.9, but not at pH 4.0. In these two soils, especially in the more acid one, *Trichoderma* and other common soil molds, such as *Mucor*, *Penicillium*, and *Fusarium*, were found to be very active. The method of Waksman[27] to determine fungi growing actively in the soil was employed in these tests. Corn meal and an agar medium containing 1 per cent glycerine only were found rather useful for these isolations.

An attempt was also made to determine the influence of variations in soil temperature on the control obtained by acidifying the surface layer of the soil. In soil-temperature tanks[16] at five temperatures between 18° C and 35° C, two pots were covered with the original soil and two pots with the original soil treated with aluminum sulfate to give a pH of 4.0 to the top 1½ inches. Three-week-old grapefruit seedlings were planted and were severely injured by the acidified soil. They were replaced by six-week-old sour-orange seedlings, a few of which were also planted in the nonacidified pots in addition to the grapefruit seedlings. All pots were uniformly inoculated with *Trichoderma* at the time of planting and with *Rhizoctonia* 3 weeks later. Complete control of the disease was obtained in the acidified series through the whole temperature range, while in the untreated set 80 to 85 per cent of grapefruit as well as sour-orange seedlings were killed by damping-off. Under these conditions no effect of soil temperature on the severity of the disease was apparent.

RELATION OF RESULTS TO BIOLOGICAL CONTROL IN GENERAL

It has been repeatedly reported that the growth of *Rhizoctonia* decreases at very acid reactions.[9, 22, 23, 29] Most of the observations have been based on measuring the diameter of cultures of *Rhizoctonia* in petri dishes. Since the fungus forms a much thinner web of hyphae in acid media than in neutral and alkaline ones, it seems probable that strong acidity diminishes the growth of the fungus even more than these data indicate. The preceding experiments, however, have shown that at the most acid reactions (pH 3.9 to 4.0) which were used, the fungus grows well enough to cause damping-off of citrus seedlings, if the sterilized soil is kept free from other organisms until the seed has germinated. The control of the disease so frequently obtained in nonsterilized acid soils without artificial introduction of other organisms may thus be due primarily to a change in the biological equilibrium of these soils caused by acidification.

This change seems to involve not only a decreased growth of *Rhizoctonia* but also an increase of the parasitic, antagonistic, and competitive activities of other organisms. *Trichoderma* spp. are reported to be very abundant in acid soils,[15, 26, 27] and under the conditions of our experiments they were found to become more active with increased acidity of the soil. The control that has been frequently obtained in combating the damping-off of pine seedlings, by acidifying the soil with aluminum sulfate or sulfuric acid,[10, 14, 32] may be due to the same factors.

A similar case has been reported with the take-all disease of wheat.[6, 7, 18, 24] The protective action of certain soils against this disease has been connected with biological factors, since sterilizing these soils destroys the biological control effect. Soil reaction does not seem to play a rôle here.

In the biological control of insect pests, the parasites are mostly obligatory, that is, they depend on the presence of hosts. The problem of biological control of soil-borne plant pathogens seems to be entirely different because the parasitic and antagonistic organisms thus far found are common soil saprophytes. There are reports on many such organisms other than *Trichoderma* that are able to act on *Rhizoctonia*.[4, 30] In the work presented here, control of the disease was not obtained by abundant inoculation of natural soils with the spores of *Trichoderma* if the soil reaction was not sufficiently acid. It seems, therefore, that primary emphasis has to be placed on creating favorable conditions for growth and antagonistic activity of the beneficial organisms, preferably conditions which also are unfavorable for the disease-inciting organism. In some cases, apparently, increase in control may be obtained by abundant inoculation with beneficial organisms, especially if the original soil flora is deficient in organisms effective under the new conditions. It is evident that fundamental studies under controlled conditions are necessary to gain a better understanding of the deciding factors in this problem. They may make it possible to develop methods of biological control and to select efficient antagonistic organisms.

SEED-BED EXPERIMENTS

Some nurserymen believe that planting the citrus seed on ridges and irrigating in furrows will keep down damping-off, since by this method the surface of the soil is kept rather dry. Others find it more practical to broadcast the seed and to water by sprinkling. The latter method was followed in two field experiments conducted in 1934 at the Cascade Ranch, San Fernando, California.[7] According to the common practice,

[7] The authors are indebted to Mr. J. T. Culbertson for his splendid coöperation, which made this work possible.

the seed was covered by a layer of sand, ⅝ to ¾ inch in depth. This sand is said to have the advantages of preventing the formation of a crust, of providing good aeration, and of keeping the surface rather dry after irrigating.

EXPERIMENT 1

The seed bed was divided into plots marked by the posts which supported the lath covering. The size of each plot was 85 square feet. The first experiment was started in the middle of April, a common time for sowing citrus seeds in this region. Twenty-three plots were planted to sweet orange, and 12 plots to sour-orange seed. The reactions to damping-off in the sweet and sour-orange plots were so similar under a given treatment that these are not separated in reporting the results. In an area that had been used during the previous year as a seed bed ("old" bed of table 1), there were 25 plots, 12 treated and 13 untreated, alternating with the others as checks. In another area of 10 plots of sour-orange seedlings, located some distance away, and not previously used as a seed bed ("new" bed), there were 6 treated and 4 check plots. This gave for the first experiment 6 plots for each of the following three treatments interspersed with 17 check plots.

Formaldehyde Dust.—A 6 per cent formaldehyde dust was mixed into the upper 3 inches of the soil 3 days before sowing, and the soil was then sprinkled lightly. The originators of this method[1] recommended 1½ ounces of dust per square foot mixed with the soil to a depth of 2½ to 3 inches. In several preliminary pot experiments, it had been found necessary to apply 3 ounces of formaldehyde dust per square foot in order to control heavy infections of *Rhizoctonia.* If used 2 or 3 days before sowing, this amount had no harmful effect on the germination of citrus seeds. It had been noted, however, that the treatment was not effective if the *Rhizoctonia* was introduced in the pots some time after sowing.

German Peat.—On the basis of the results of the greenhouse experiments reported above, the seed was planted between two layers of peat moss, each ¾ inch deep. Simultaneously with the planting, the peat was inoculated with giant cultures of *Trichoderma* of three types: (1) pigmented cultures of *T. lignorum,* (2) "odorous" cultures of *T. lignorum,* and (3) *T. koningi.* The *Trichoderma* cultures had been grown for 1 week on peat which was saturated with a glycerine medium.

Formaldehyde Dust and Peat.—This is a combination of treatments 1 and 2. Only half the amount of formaldehyde was applied, and the seed was covered but not underlaid with peat moss.

Results of Treatments in Experiment 1.—The reaction of the soil,

which has the nature of a Hanford sandy loam, varied from pH 6.6 to 6.7, while that of the sand cover was from pH 7.6 to 7.9. These reactions remained practically the same in the check plots during the period of damping-off. In the peat moss, however, there was a change of the pH from 3.9–4.0 to 4.5–4.6 after 6 weeks and to 4.9–5.5 after 14 weeks. In all plots treated with peat about three-fifths of the area in which the seed-

TABLE 1

EFFECT OF FORMALDEHYDE DUST AND PEAT SEED-BED TREATMENT ON RHIZOCTONIA DAMPING-OFF OF CITRUS SEEDLINGS IN EXPERIMENT 1

(Planted April, 1934; results noted in October, 1934)

Treatment	Number of plots, 85 square feet each	Area affected with damping-off*		
		Total square inches	Square inches per plot	Per cent
New seed bed				
Check	4	6,399	1,600	13.0
Formaldehyde dust	2	2,731	1,365	11.1
Formaldehyde dust and peat cover	2	1,769	884	7.2
Peat	2	253	126	1.0
Old seed bed				
Check	13	11,507	885	7.2
Formaldehyde dust	4	8,680	2,170	17.7
Formaldehyde dust and peat cover	4	1,012	253	2.1
Peat	4	19	5	0.05

* Damping-off rather uniformly distributed in new bed but very unevenly in old seed bed.

lings were attacked by the fungus showed the disease more than three and a half months after sowing. In the other plots practically all of the damping-off occurred in the first two or three months, most of it in the first 6 weeks. This coincidence of decrease in acidity of the peat with late damping-off is very suggestive, for it corresponds to our results in the experiments reported above.

As most of the damping-off developed in circular spots, the areas which were destroyed could be easily calculated from the diameters measured. Dead seedlings and attacked plants with dark-brown lesions at the stem base mark the margin of the areas. Table 1 represents the results six months after the planting of the seeds.

It should be emphasized that the areas affected with damping-off were rather evenly distributed over the 10 plots of sour-orange seedlings (new seed bed). In the other bed (old bed), most of the disease was in one section of the bed containing 2 formaldehyde-dust-treated plots. The series with the 10 plots gives, therefore, a truer picture of the actual situation.

EXPERIMENT 2

A second experiment was started two months after the first. Thirteen plots adjacent to row 2 of the first experiment were planted with sweet orange, and, in addition, nearly 4 plots in the next row were planted with grapefruit and 1 with rough-lemon seed. Again each alternate plot was left untreated. The peat treatment had shown much promise in controlling the disease, so it was used again. As a second treatment, aluminum sulfate was applied at the rate of 35 grams per square foot. It was spread over the surface just before sowing, and raked into the top inch of the soil. The third treatment was a combination of the first two, the soil being treated with aluminum sulfate at the rate of 25 grams per square foot, and the seed covered but not underlaid with peat. The peat was inoculated with beneficial fungi as previously; to the soil treated with aluminum sulfate, fungi were not added.

TABLE 2

Effect of Aluminum Sulfate and Peat Seed-Bed Treatment on Rhizoctonia Damping-Off of Citrus Seedlings in Experiment 2

(Planted June, 1934; results noted in December, 1934)

Kind of seedlings	Treatment	Number of plots 85 square feet each	Area affected with damping-off*: Total square inches	Square inches per plot	Per cent
Sweet orange	Check	7	26,603	3,800	31.0
	Aluminum sulfate	2	417	208	1.7
	Aluminum sulfate and peat cover	2	716	358	2.9
	Peat	2	1,004	502	4.1
Grapefruit	Check	2¾	12,097	4,400	35.9
	Aluminum sulfate and peat cover	1	330	330	2.7
Rough lemon	Peat	1	237	237	1.9

* Damping-off uniformly distributed.

Results of Treatments in Experiment 2.—The aluminum sulfate made the reaction of the upper soil inch much more acid (pH 3.5 to 3.6) than was expected from preliminary tests. The reaction of this soil was pH 4.0 after 6 weeks, and pH 4.7 to 4.9 after 4 months, while that of the peat become pH 5.0, and the soil below the peat had a reaction of pH 5.6. There was some delay of germination in plots treated with aluminum sulfate, but later the seedlings seemed to catch up with those in the check plots. The more frequent watering necessitated by the higher tempera-

tures after the late sowing may have contributed to the higher percentage of damping-off in the second experiment (table 2).

In the treated plots, the damping-off again developed later than in the checks, that is, two or three months after sowing. The very effective control of the disease obtained by the acidification treatments in the second experiment offset or at least masked any detrimental effect on the growth of the seedlings.

EFFECT OF ACIDIFICATION ON GROWTH OF SEEDLINGS

In the first experiment, there was a definite stunting effect discernible in the peat-treated plots. The average size, vigor, and stem diameter of the seedlings were evidently decreased in comparison with the adjoining check plots. It is thought that relatively lower soil temperature in and under the peat may be one of the factors that contribute to a slower growth of the seedlings, in addition to the temporary effect of excessive acidity on the root development of citrus seedlings, the unfavorable influence of which has been reported.[8]

There had been previously observed in greenhouse experiments certain detrimental effects of peat on sweet-orange seedlings. The roots of some seedlings developed poorly or were stunted. It had been expected that in the field the plants would reach the less acid subsoil soon and would overcome any detrimental effect easily. In fact the contrary was true, perhaps because the frequent watering in the greenhouse brought about a much more rapid decrease of acidity in the peat than would appear in the field.

An attempt was made to remedy the stunting effect of the peat. In addition to the fertilization of the checks with dried blood and calcium nitrate, when the seedlings were six months old the following three corrective treatments were tried in halves of the treated plots, the other halves receiving calcium nitrate only: (1) 11 pounds of quicklime per 100 square feet; or (2) 1 pound of bordeaux powder per 100 square feet; or (3) 2 pounds of a zinc sulfate and lime powder containing equal parts of lime and $ZnSO_4$ per 100 square feet. None of these treatments showed any considerable improvement over the checks.

Thus, in spite of the good control of damping-off obtained, the acidifying methods employed here, especially the peat treatment, cannot be recommended yet for general use on account of the stunting effect on the seedlings. The most promising of the treatments so far seems to be the aluminum sulfate.[8] It is more adaptable to modification than the peat

[8] As this goes to press the results of 1935 experiments with 30 grams of aluminum sulfate per square foot have shown only 2 per cent damping-off against 39 per cent in adjacent checks, and no stunting effect on the seedlings.

treatment, which is inconvenient to apply and seems to keep the soil too cool for the best root growth. The value of adding beneficial fungi with the acidifying treatments remains to be seen through future investigation. Increased control was obtained in some greenhouse experiments. No comparable experiments were made in the field.

SUMMARY

Damping-off of citrus seedlings, caused by *Rhizoctonia solani*, was successfully controlled in laboratory, greenhouse, and field experiments by acidifying the soil layers next to the seed by the application of aluminum sulfate or acid peat moss, which produced an initial reaction of about pH 4.0.

However, damping-off was not controlled in sterilized soils of the same acidity in the absence of *Trichoderma* spp. Therefore, the control of the disease by the acidification of nonsterile soil cannot be explained entirely on the grounds that such a medium is unfavorable to the growth of *Rhizoctonia*. Evidence is given which indicates that the decisive factor is a change in the microflora of the soil, favoring organisms such as *Trichoderma*, which may be antagonistic or parasitic towards *Rhizoctonia solani*.

In some cases, with moderately acidified natural soils, abundant inoculation with *Trichoderma* spp. was accompanied by a larger percentage of healthy seedlings. Such biological control was entirely absent in soil of neutral reactions.

Growing seedlings at a series of constant soil temperatures from 18° to 35° C seemed to affect neither the severity of damping-off due to *Rhizoctonia solani* nor the degree of control of the disease through applying aluminum sulfate.

The addition of peat moss to the seed bed proved unsatisfactory from the commercial standpoint, since it had a stunting effect on most of the seedlings. Treatment with aluminum sulfate appears promising as a practical method for controlling the damping-off disease of citrus seedlings.

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INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF TURKEY WHEATS C. I. 1558B AND C. I. 2578

FRED N. BRIGGS

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF TURKEY WHEATS C. I. 1558B AND C. I. 2578[1, 2]

FRED N. BRIGGS[3]

INTRODUCTION

TURKEY is the name most commonly applied to the Crimean group of hard red winter wheats grown in the United States. In 1924, according to Clark and his co-workers,[11] the hard red winter wheats comprised 41.4 per cent of the total wheat acreage in this country; and Turkey, including Kanred, made up 91.7 per cent of the acreage devoted to hard red winter wheat. At that time, therefore, over 36 per cent of the entire wheat acreage was devoted to Turkey. This type of wheat was first brought to the United States in 1873 and was grown in Kansas.[8] Since that time numerous introductions have been made both by private and by public agencies. Other names that have been applied to the type are Alberta Red, Argentine, Bulgarian, Crimean, Defiance, Egyptian, Hard Winter, Hundred-and-One, Hungarian, Improved Turkey, Kharkoff, Lost Freight, Malcome, Malakof, Minnesota Red Cross, Minnesota Reliable, Pioneer Turkey, Red Russian, Red Winter, Romanella, Russian, Taurenian, Theiss, Turkey Red, Turkish Red, Ulta, Wisconsin No. 18, and World's Champion.

Recently certain strains of Turkey wheat have been distributed under other varietal names based on performance records and slight morphological differences. It has been long recognized that there are both morphological and physiological differences between certain of these Turkey strains. Sherman[9] and Oro[10] are two such wheats.

The Turkey wheats have been an important source of varieties resistant to bunt (*Tilletia tritici*), for genetic studies and for the production of other resistant varieties. Of the 17 most resistant varieties discovered by Tisdale and his co-workers,[14] 11 were Turkey wheats. Two others, Banner Berkeley and Ridit, resulted from hybrids that had Turkey for

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[3] Assistant Professor of Agronomy and Assistant Agronomist in the Experiment Station.

one parent. Kiesselbach and Anderson[12] isolated 12 resistant lines from Turkey (South Dakota 144) wheat. Other lines showed varying amounts of smut up to more than 90 per cent.

The genetics of bunt resistance has been studied by the present writer[7] in 9 resistant varieties of wheat. Four of these—Turkey 1558, Turkey 3055, Sherman, and Oro—have been Turkey types. Two of the three genetic factors found are represented in these wheats. Sherman has the Martin factor,[7] whereas Oro, Turkey 1558, and Turkey 3055 have the Turkey factor.[7]

TABLE 1

Annual Percentages of Bunt Infection at Davis, California, in the Parent Wheat Varieties During the Years Indicated

Parent variety	Percentage of bunted plants						
	1929	1930	1931	1932	1933	1934	Average
Turkey 1558B	0.0	1.1	0.0	1.9	0.0	0.8	0.6
Turkey 2578	0.0	2.9	1.9	0.0	0.0	1.3	1.0
Turkey 3055	0.1	2.0	1.2	1.8	0.1	0.3	0.9
Martin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Selection 1403	....	0.0	0.0	0.0	0.0	1.4	0.3
Baart	....	....	47.2	85.5	66.0	84.4	70.8
White Federation	78.6	59.3	43.0	73.2	59.9	73.7	64.7

The inheritance of resistance to bunt has been studied in hybrids involving two other resistant varieties of the Turkey type—namely, Turkey 1558B and Turkey 2578. The former is from a pure-line selection made at Moro, Oregon, by Carleton R. Ball. The history of Turkey 2578 is unknown to the present writer.

EXPERIMENTAL RESULTS

The parental material and hybrid populations were grown in the field at the University Farm, Davis, California. The methods of handling and the inoculum used have been described in previous publications.[2-6] The collection of bunt has been designated as physiologic race III of *Tilletia tritici* by Reed[13] and physiologic form VIII by Bressman.[1]

The percentage of bunt infection in the parent varieties may be seen in table 1.

The percentage of bunted plants in each case is based on 2 or more rod rows. Usually 30 or more rows of the susceptible parent were grown, and frequently 10 or more rows of the resistant parent were included. There were 30 to 70 plants per row. The difference between resistant and susceptible parents is very marked.

Turkey 1558B and Turkey 2578 were crossed with Baart to determine

the number of bunt-resistant factors in each of these two resistant varieties. Crosses using these were also made with Martin, Turkey 3055, and Selection 1403 to test for the presence of the Martin, Turkey, and Hussar factor respectively. The last-named cross was grown only in F_2.

F_1 seeds were not inoculated, because of the small number available. Where F_2 plants were being grown as a source of seed to be inoculated in F_3, they were kept free from bunt in order to prevent the elimination of susceptible progeny by bunt in F_2.

TABLE 2

PERCENTAGE OF BUNTED PLANTS IN THE PARENTS AND IN F_2 OF THE CROSSES NAMED; DAVIS, CALIFORNIA, 1934

Parent or cross	Number of plants grown	Number of plants bunted	Per cent of plants bunted
Turkey 1558B	1,276	12	0.9
Baart	2,155	1,822	84.5
Turkey 2578	1,197	17	1.4
Martin	432	0	0.0
Selection 1403	345	5	1.4
Turkey	391	1	0.3
Turkey 1558B × Baart	711	236	33.2
Turkey 1558B × Martin	1,066	71	6.7
Turkey 1558B × Turkey 3055	389	4	1.0
Selection 1403 × Turkey 1558B	481	61	12.7
Turkey 2578 × Baart	827	310	37.5
Turkey 2578 × Martin	825	33	4.0
Turkey 2578 × Turkey 3055	811	27	3.3
Selection 1403 × Turkey 2578	501	48	9.6

F_2 populations of all the crosses were inoculated and grown in 1934 along with the F_3. Although F_2 data do not permit a complete Mendelian analysis, they do indicate the number of resistant factors present as well as their identity and effect.

The F_2 data, accordingly, are also included in table 2.

The classification of the F_2 plants on the basis of the bunt obtained in F_3 rows gives much more satisfactory data. These rows contained from 30 to 70 plants, usually about 50. In most cases this classification is certain and reliable. Concerning a few rows that fall near the minima there is some uncertainty; but these rows are relatively few (table 3).

The rows in the 0–5 per cent class for bunt infection were subdivided into those without any bunt and those with 1–5 per cent.

The hybrids with Turkey 1558B may be considered first. The distribution of rows in Turkey 1558B × Baart is shown in figure 1. The number of rows under the three modes is very near the 1:2:1 ratio. Accepting 12.5 and 47.5 as minima, there are 60 resistant, 123 segregating, and 62 sus-

TABLE 3

DISTRIBUTION OF PARENT AND F_3 ROWS OF THE CROSSES NAMED INTO 5 PER CENT CLASSES FOR BUNT INFECTION; DAVIS, CALIFORNIA, 1934

Parent or cross	Distribution of rows having a bunt infection (per cent) of — 0–5: 0	0–5: 1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	Total number of rows
Turkey 1558B	8	8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	16
Turkey 2578	7	9	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	16
Baart	....	....	....	....	....	....	....	....	....	....	....	....	....	....	2	1	6	8	18	6	1	42
Martin	8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	8
Turkey 3055	7	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	8
Turkey 1558B × Baart	7	29	18	12	13	27	30	21	18	6	4	7	6	10	8	12	8	6	3	....	....	245
Turkey 1558B × Turkey 3055	52	40	16	3	1	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	113
Martin × Turkey 1558B	50	23	16	8	7	5	3	1	1	0	1	2	0	0	0	1	....	....	....	....	....	118
Turkey 2578 × Baart	14	21	22	8	11	20	20	30	19	18	9	11	10	5	14	8	6	7	2	....	....	255
Turkey 2578 × Turkey 3055	69	37	10	3	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	121
Martin × Turkey 2578	48	24	21	16	1	2	0	1	0	3	0	1	1	0	0	1	....	....	....	....	....	119

ceptible rows where 61.25, 122.5, and 61.25 are the numbers expected. Turkey 1558B therefore differs from Baart in one major factor for resistance to bunt. Both F_2 and F_3 data indicate that resistance is incompletely dominant.

The identity of the factor for resistance to bunt in Turkey 1558B is established by the cross with Turkey 3055, the tester for the Turkey factor. No susceptible rows occurred in a population of 113 rows which

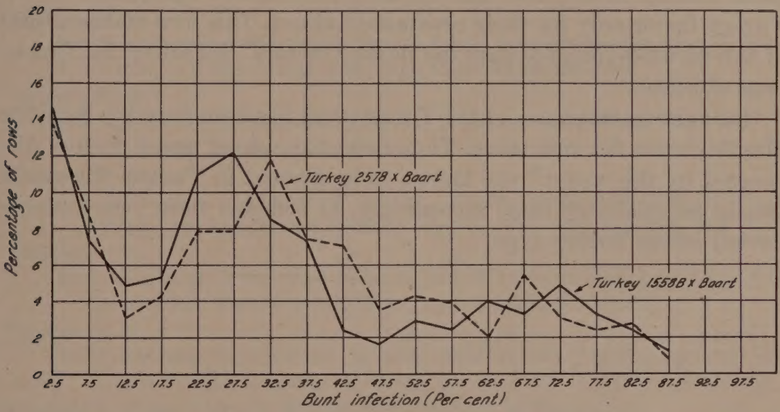

Fig. 1.—Distribution of F_3 rows of Turkey 1558B × Baart and Turkey 2578 × Baart into 5 per cent classes of bunt infection.

shows that Turkey 1558B is identical with Turkey 3055 as regards its major factor for resistance to bunt and therefore has the Turkey factor for resistance.

The hybrids with Turkey 2578 may now be considered briefly. The distribution of rows in Turkey 2578 × Baart (fig. 1) resembles that of Turkey 1558B × Baart. If we accept 12.5 and 47.5 as minima, the agreement with the 1 :2 :1 ratio is very close. There is not a very clear-cut minimum at 47.5. In practically all other crosses between resistant and susceptible varieties, however, there has been a fairly well-defined minimum in this region. If the susceptible and segregating rows are added together, a very good 3:1 ratio is obtained. Further evidence that the resistance of Turkey 2578 results from a single factor is furnished by the cross with Martin. There are 3 susceptible rows in a population of 119—a satisfactory agreement with the 15:1 ratio. The value of P is between 0.1 and 0.2. The segregation is similar to that obtained in the cross of Martin × Turkey 1558B and in other crosses where the Martin and Turkey factors were present. In view of these considerations, the

data indicate that Turkey 2578 differs from Baart in one major factor for resistance to bunt. Here again, resistance is incompletely dominant.

The identity of the factor for resistance to bunt in Turkey 2578 is established by the cross with Turkey 3055. There were no susceptible rows in a population of 121 rows.

DISCUSSION AND SUMMARY

Both Turkey 1558B and Turkey 2578 were found to depend on the Turkey factor only for their resistance to bunt. This now makes a total of five varieties found to have the Turkey factor.[7] All are of the Turkey type of wheat.

One variety, Sherman, of the Turkey type has been found to have the Martin factor for resistance. Thus two of the three major factors discovered by the writer[7] are known to be present in Turkey wheats. It should be relatively easy, accordingly, to combine these into a single variety of the Turkey type.

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